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Brief Clinical Report

Pallister-Killian Syndrome: A Mild Case Diagnosed by Fluorescence In Situ Hybridization. Review of the Literature and Expansion of the Phenotype

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Pallister-Killian syndrome (PKS) is a rare disorder characterized by a specific combination of anomalies, mental retardation and mosaic presence of a supernumerary isochromosome 12p which is tissue-limited. We report an atypical case of PKS with a mild phenotype. Fluorescence in situ hybridization (FISH) was used to demonstrate that the supernumerary marker chromosome identified in the patient's fibroblasts was an isochromosome 12p. This study broadens the spectrum of PKS phenotype. It also illustrates the usefulness of fluorescence in situ hybridization in diagnosis of patients with chromosomal abnormalities and mild or atypical clinical findings.

KEY WORDS: Pallister-Killian syndrome, isochromosome, chromosome 12, fluorescence in situ hybridization, fibroblasts, hemihypertrophy

INTRODUCTION

Pallister-Killian syndrome (PKS) is a rare and sporadic syndrome first described in adults by Pallister et al. [1977]. Since the original description of the patients of Pallister et al. [1977] over 60 cases of PKS including children, infants and those ascertained through prenatal diagnosis have been reported. Reynolds et al. [1987] summarized the manifestations of PKS in 11 patients. Detailed clinical findings of PKS have also been reported by Bergoffen et al. [1993], Gamal et al. [1994], Gilgenkrantz et al. [1985], Kwee et al. [1984], McPherson et al. [1993], and Schinzel [1991]. Individuals with PKS generally show a "coarse" face, a broad high forehead, malformed ears, macrostomia, a broad nose and hypertelorism. Neurological manifestations include profound mental retardation, seizures and hypotonia. There are peculiar pigmentary skin anomalies. Most of the patients have sparse hair and eyebrows, and many have various internal structural abnormalities. Other common manifestations are listed in Table I.

Individuals with PKS have an aneuploid cell line which contains a supernumerary isochromosome 12p, i(12p). Sometimes the i(12p) was misinterpreted as i(21q) [Hunter et al., 1982; Lopes et al., 1985; Kwee et al., 1984; Fryns et al., 1982] and in one case tetrasomy 12p was misinterpreted as trisomy of chromosome 20 [Pan et al., 1976]. The presence of the i(12p) in PKS is mosaic and tissue-specific. It is found in high percentage in skin fibroblasts [Kawashima, 1987; Narahara et al., 1988; Peltomaki et al., 1987; Priest et al., 1992; Reynolds et al., 1987; Soukup and Neidich, 1990; Speleman et al., 1991; Tejada et al., 1992], amniocytes, and bone marrow cells [Reeser and Wenger, 1992; Von Koskull et al., 1989; Ward et al., 1988]. However, the isochromosome is absent in lymphocytes [Mclean et al., 1992; Narahara et al., 1988; Ohashi et al., 1993, Priest et al., 1992; Soukup and Neidich, 1990; Warburton et al., 1987] or present only at a very low frequency [Pallister et al., 1977; Pauli et al., 1987; Peltomaki et al., 1987; Reynolds et al., 1987; Reeser and Wenger, 1992; von Koskull et al., 1989]. The percentage of aneuploid cells does not correspond to the severity of the phenotype. The presence of the extra chromosome decreases with advancing age and might therefore be age-related in vivo. In vitro, the frequency of cells with the extra chromosome is influenced by "passage" of the cell culture [Gamal et al., 1994; Peltomaki et al., 1987; Priest et al., 1992; Quarrell et al., 1988; Ward et al., 1988; Speleman et al., 1991; Warburton et al., 1987].

Most PKS patients share the findings summarized by Reynolds et al. [1987]. However, the abnormalities are sometimes mild [Pan et al., 1976; Wilson et al., 1994; Lopes et al., 1985; Zakowski et al., 1992; El-Naggar and Hawthorne, 1994], or overlap with those of other syndromes [Rodriguez et al., 1994; McPherson et al., 1993;

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TABLE I. Frequency of Commonly Reported Pallister-Killian Clinical Findings in Comparison to the Clinical Findings of Our Patient*

	Frequency in previously reported cases (n = 67) (ratio M/F[a] = 32/35)	Present case (M)
Internal structural abnormalities	46	–
Abnormal ears	46	–
Hypertelorism	41	+
Coarse face	38	–
Broad bridge of nose	36	+
High broad forehead	35	–
Hypotonia	35	+
Epicanthal folds	33	–
Bitemporal alopecia	31	+
Pigmentary dysplasia	29	+
Seizures	28	–
Severe mental retardation	26	–
Macrostomia	25	–
Small upturned nares	25	–
Sparse scalp hair	23	+
Short limbs	20	–
Finger anomaly	19	–
Short neck	19	–
Webbed neck	18	–
Prominent upper lip	17	–
Sparse eyebrows	16	+
Macroglossia	14	–
Anal abnormalities	14	–
Abnormal genitalia	13	–
Accessory nipples	9	–
Hearing loss	6	+
Hemihypertrophy of the body	0	+

* Eleven of the cases were taken from a review by Reynolds et al. [1987]. The remainder were taken from literature cases cited in the references.
[a] M, male; F, female.

Buyse and Korf, 1983; Stengel-Rutkowski et al., 1981]. In such cases, it is particularly important that the cytogenetic diagnosis be established.

In this study we report on a patient who, due to dyspigmented skin, and only a few other clinical abnormalities, was initially diagnosed with hypomelanosis of Ito. The patient's lymphocyte chromosomes were normal but his fibroblast karyotype demonstrated the presence of a supernumerary marker chromosome. This marker was characterized as i(12p) by fluorescence in situ hybridization (FISH) using chromosome 12 centromeric and total chromosome 12 probes, i.e., by chromosome "painting."

MATERIALS AND METHODS

Clinical Report

K.M. was born at 38 weeks of gestation to a 33-year-old mother and a 31-year-old father who were healthy and unrelated. At birth he was found to have swirly brown pigmented spots over most of the body. On initial examination at age 3 years the patient showed unusual hair growth, hypopigmentated spots on the face, trunk and lower limbs (Fig. 1). He also had a cupid's bow upper lip, a scaphocephalic skull shape with a prominent forehead, a flat nasal bridge, sparse eyebrows, and mild hypotonia. There was a moderate delay in his gross motor development. Social and cognitive skills were normal for the chronological age. Abdominal ultrasound, cardiac examination, and MRI of the head showed no abnormalities. Analysis of peripheral lymphocytes showed a normal 46,XY karyotype. A diagnosis of hypomelanosis of Ito was suggested. Analysis of the patient's fibroblasts showed a supernumerary marker chromosome in ~80% of cells analyzed (46,XY/47,XY,+mar). Based on the banding pattern the marker was interpreted as either an i(21q) or an i(12p).

At age 5 years (Fig. 1) the patient was reevaluated. His height was 95 cm (just below 25th centile) and weight was 15.5 kg (<50th centile) and his OFC was 50 cm (<50th centile). He still had the same abnormal pigmentation and a sweeping hair pattern (Fig. 1). A frenulum was noticed between the gingival margin to the upper lip. He had esotropia of the right eye, and a mild myopia of the left eye. His lenses were clear. A hearing test indicated sensory neural hearing loss in the right ear. Measurements of arm and leg circumference indicated larger values of the right side of the body. There was no obvious accessory nipple. Developmental evaluation showed scattered skills with a delay mainly in speech and communication skills. The patient demonstrated good memory and social skills.

FISH

All analyses described were performed at age 5 on fibroblasts obtained from a biopsy of a hypopigmented skin lesion. The FISH method used was based on the ONCOR protocol. The 2 probes used for hybridization were a chromosome 12-specific α-satellite probe D12Z1

Fig. 1. Patient at age 5. Note prominent forehead, flat nasal bridge, sparse eyebrows, bitemporal alopecia, and hypopigmentation of the face and trunk.

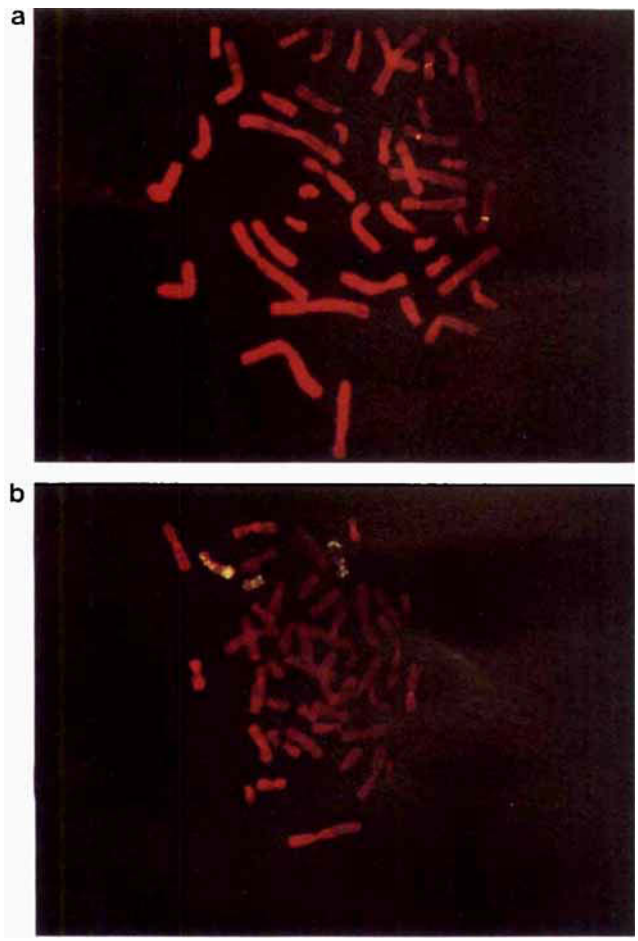

Fig. 2. **a:** Photomicrograph of a metaphase after FISH with chromosome 12 centromeric probe showing signal on centromeres of two normal chromosomes 12 and on the centromere of the marker. **b:** Photomicrograph of a metaphase after FISH with total chromosome 12 probe showing signal on two chromosomes 12 and on isochromosome 12p.

(ONCOR, Gaithersburg, MD) and a COATASOME™ total chromosome 12 probe (ONCOR). The chromosomes were viewed using a Leitz epifluorescence microscope. Images were recorded using image analysis software (Applied Imaging, Cytovision, Pittsburgh, PA).

RESULTS

FISH with the α-satellite probe resulted in strong hybridization signals in metaphase and interphase cells. Essentially all aneuploid metaphases exhibited signals over the centromeric regions of the chromosome 12 homologs and of the marker (Fig. 2a). The percent of interphase cells with 3 signals (55%) corresponded to the frequency of aneuploid cells, as assessed by metaphase analysis (75%).

FISH with the total chromosome 12 probe was done on "passage 6" fibroblasts. The technique gave a very high efficiency which +was independent of the degree of spreading and morphology of the chromosomes. Hybridization resulted in bright but uneven signals spanning the length of chromosome 12 and both arms of the marker, when present (Fig. 2b).

DISCUSSION

There are over 67 reported cases of PKS. The clinical manifestations associated with PKS are usually severe. The most common abnormalities in young children are mental retardation, seizures, hypotonia, "coarse" face, flat nasal bridge, broad forehead, bitemporal alopecia, abnormal ears, hypertelorism, pigmentary dysplasia, short limbs and finger anomalies. Our patient had only a number of the findings consistent with the syndrome (Table I) and some findings overlapped with those of other syndromes such as Fryns syndrome, trisomy 12p, and hypomelanosis of Ito. The patient had hearing loss, reported in only 6 other PKS cases [Speleman et al., 1991; El-Naggar and Hawthorne, 1994; Pagon, 1983; Hersh et al., 1983; Lubinsky, 1993; Horn et al., 1995].

Our patient also had right hemihypertrophy of the body which to our knowledge, has not been reported previously in this syndrome. In this case the hemihypertrophy may be associated with a postzygotic event generating the abnormal cell line.

Pallister-Killian syndrome is associated with the mosaic presence of a supernumerary 12p. Thus, individuals with atypical, or mild phenotype of PKS must be identified by this cytogenetic criterion. The presence of the i(12p) can be determined by routine cytogenetic analysis. Our subject had a supernumerary marker chromosome in his fibroblasts; however, due to the patient's atypical phenotype the identity of the marker had to be confirmed by molecular cytogenetics.

PKS cases have been confirmed by FISH using chromosome 12 centromeric probes [e.g., Blancato et al., 1992] and total chromosome 12 probes or "paint" [e.g., Bernert et al., 1992; Larramendy et al., 1993]. Our patient is the tenth documented to be diagnosed by this method. We excluded the involvement of other chromosomes in the generation of the supernumerary marker by chromosome "painting."

The case described in this report broadens the spectrum of the Pallister-Killian syndrome phenotype and illustrates that mild forms of this disorder exist. Consequently, it raises some important questions regarding counselling following prenatal detection of supernumerary i(12p). Our findings also suggest that one ought to consider karyotyping skin fibroblasts from individuals with pigmentary dysplasia and mild delay and dysmorphic features.

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